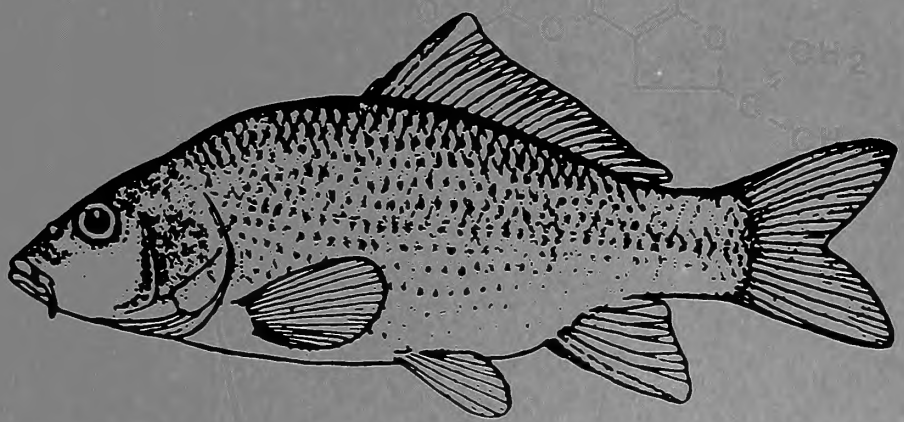
INVESTIGATIONS IN FISH CONTROL

98. History of Acute Toxicity Tests with Fish, 1863–1987



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1989

History of Acute Toxicity Tests with Fish, 1863–1987

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Abstract

Acute toxicity tests with fish were first reported in 1863. Test methodology developed slowly before World War II, during the period when the goldfish (*Carassius auratus*) was the recommended test animal. After the war, considerable effort was made to standardize test methods. A standardized method was published in 1960, refined in 1975, and updated in 1980. The primary use of the static acute toxicity test is to determine the median concentrations of chemicals that are lethal to aquatic organisms. Other applications include establishing the half-life of the biological activity of chemicals, evaluating the toxicity of mixtures of chemicals, and developing quantitative structure–activity relations used in predicting the toxicity of particular groups of chemicals. Data from acute toxicity tests are now required for the registration of chemicals for use in fishery work by the U.S. Food and Drug Administration and the Environmental Protection Agency. Acute toxicity data are also used in ecological risk assessment. Static acute toxicity tests will continue to play an essential role in aquatic toxicology in the foreseeable future.

Although the history of water pollution research was discussed by Hynes (1960), Warren (1971), and Tarzwell (1978), only limited attention has been given to the use of fish in acute toxicity tests. Since little historical background is given in recent texts on aquatic toxicology (Nriagu 1983; Rand and Petrocelli 1985), the present review is intended to be an introduction to toxicity testing or of interest to those seeking background information on acute toxicity tests. Certain persons and events are highlighted because of their importance in the development of test techniques or contributions to the evolution of acute toxicity testing. Toxicity testing with fish is briefly summarized later, in the Table.

Statistical procedures used in evaluating aquatic toxicity tests are not discussed. Statistical approaches used to estimate toxicity in acute tests were given by Brown (1973), Stephan (1977), and Gelber et al. (1985).

In much of the early literature on pollution studies with fish, the term "bioassay" described what is now termed a "toxicity test." These two terms, as defined by Rand and Petrocelli (1985), are now distinct: A **bioassay** is used to evaluate the relative potency of a chemical by comparing its effect on a living organism with the effect of a standard preparation (e.g., a reference hormone or toxicant) on the same type of organism; a **toxicity test** is the means used to determine the toxicity of a chemical or other test material.

The Early Years, 1863–1939

Fishes have been used in experiments for nearly 200 years. Early research included studies of experimental embryology and parasitology, genetics, nutrition, renal physiology, endocrinology, and nerve physiology (Nigrelli 1953). One of the first reports of the use of fish in toxicity studies was that of Penny and Adams (1863). Laboratory tests were performed on the effects of chemicals (used in dye-works) to two fishes—a minnow because it was sensitive to "disturbing influences" and the goldfish (*Carassius auratus*) because of its "tenacity for life." Penny and Adams conducted more than 400 ex-

periments with 71 chemicals. Water for testing came from the River Leven, and toxicants were added in the laboratory. Powers (1917) published a monograph proposing the goldfish as a test animal in toxicity studies and introduced the concept of measuring survival time versus concentration of the test chemical. This basic concept is still used in some applications such as time to 50% mortality and lethal threshold concentration (Sprague 1973).

The effect of the mining of lead (Pb) on stream fauna and flora was studied in England during the 1920's. Carpenter (1925) was one of the first investigators to use caged fishes to determine the toxicity of runoff from mine tailings and to conduct laboratory tests with river water to examine the toxicity of Pb to the minnow *Leuciscus phoxinus* and to goldfish. Parallel laboratory studies were also conducted at concentrations similar to those measured in the field (Carpenter 1925). Signs of Pb poisoning, such as increased mucus production and decreased survival time at those Pb concentrations, supported the inference that the mortality observed in the field was due to Pb poisoning. Additional studies with 13 other species of freshwater fishes showed that the exposure of fish to Pb salts in distilled water caused similar signs of intoxication (Carpenter 1930). Carpenter also noted that the introduction of small amounts of hard water into test vessels containing distilled water as the test medium caused erratic results in Pb toxicity tests.

Belding (1927) provided an excellent early critical review of conditions necessary for the use of fish in the successful testing of polluting substances. He identified important variables that concerned the test animal and dilution water. The important test variables related to fish included: individual variation; differences among families or species of fishes; differences in age, size, weight, and vitality; and differences in the environment (water quality, number of fish per container, dissolved oxygen, temperature). Six characteristic signs of poisoning—irritation, inactivity, erratic swimming behavior, oxygen hunger, loss of equilibrium, and increased or decreased respiration rate—were suggested for use in documenting the toxicity of the test substances. Steinmann (1928) was the first to measure both heart rates and respiration rates of fish exposed to chemicals. Interest in relating biochemical and physiological responses to chemical exposure later developed into sophisticated approaches for defining acute toxicity syndromes in fish (McKim et al. 1987).

The first detailed investigation that combined field and laboratory studies on the toxicity of pollutants to fishes was conducted by Ellis (1937). His work was notable for three reasons: (1) he conducted extensive field studies to define water quality characteristics associated with healthy aquatic communities, including some recommended water quality values such as dissolved oxygen concentration and pH range that remain applicable today; (2) he compiled the known modes of action and toxicity of 114 toxic substances; and (3) he and his colleagues performed many laboratory toxicity tests with goldfish and *Daphnia magna* in soft and hard waters. He also established hazard rankings to fishes (none, possible, moderate, and critical) for 30 common types of municipal and industrial effluents.

Many early investigators used distilled water as the test medium to reduce possible contamination with metals; however, its use was later found to impart an additional osmotic stress to freshwater fishes (Hunn and Allen 1964). In experiments with the threespine stickleback (*Gasterosteus aculeatus*), Jones (1938) noted that Pb was far less toxic in hard tap water containing 50 mg/L of calcium than in soft tap water containing only 1 mg/L. Further studies with test waters containing calcium that ranged from 0 to 50 mg/L (as calcium chloride or calcium nitrate) documented the ameliorating effects of calcium on Pb toxicity, which included reductions in respiratory distress and in the accumulation of coagulated mucus. Later studies with goldfish and with mucus from the eel *Anguilla anguilla* showed that excess coagulated mucus was not formed in the presence of sufficient calcium; however, if Pb first interacted with the mucus to cause coagulation, the addition of calcium did not reverse the process, because Pb has a greater affinity than calcium for binding sites on mucus.

Early observations by Carpenter (1930) and Jones (1938) on the effects of hardness or calcium on the toxicity of divalent metals ultimately led to water hardness criteria for certain metals. For example, criteria for cadmium and lead are based on hardness; lower allowable concentrations were suggested for soft water than for hard water (Thurston et al. 1979).

The Middle Years, 1940–1960

A new era in aquatic toxicology in the 1940's started with the advent of organochlorine insecticides (Dunlap 1981). To study problems created by these compounds and other pollutants in fresh water, the U.S. Public Health Service established the Aquatic Biology Section at its laboratory at Cincinnati, Ohio, in 1948 (Tarzwell 1978). The next 10 years introduced the era of the "pickle-jar biologist," as the 5-gallon pickle jar became the standard vessel for acute toxicity tests (Fig. 1). Accompanying the expansion of research into pollution problems and the need to establish water quality criteria, two extensive critical

Fig. 1. Making observations during a static acute toxicity test in the widely-used 5-gallon pickle jars of 1940–60 at the Fish Control Laboratory, LaCrosse, Wisconsin. *Photo by W. Mauck.*

reviews of the toxicity of industrial wastes and their components to fish were published by Doudoroff and Katz (1950, 1953). During this period numerous toxicity studies with freshwater fishes were being conducted at the U.S. Fish and Wildlife Service Laboratory, Leetown, West Virginia (Wood 1953; Hollis and Lennon 1954). More than 4,000 chemicals were screened for their toxicity to six species of fish: brook trout, *Salvelinus fontinalis*; brown trout, *Salmo trutta*; rainbow trout, *S. gairdneri*; bluegill, *Lepomis macrochirus*; yellow perch, *Perca flavescens*; and goldfish.

Another large-scale toxicity testing program began in 1953 at the Hammond Bay Biological Station of the U.S. Fish and Wildlife Service, in a search for a selective toxicant to control the sea lamprey (*Petromyzon marinus*) in the Great Lakes. The testing of more than 4,000 chemicals led to the discovery of the selective larval lampricide 3-trifluoromethyl-4-nitrophenol (Applegate et al. 1957; Howell et al. 1980).

Soon after World War II, a major element in the development of toxicity testing was a drive to standardize test methods. One early attempt (Hart et al. 1945) was limited in scope and effect because only 300 copies of a manual of suggested procedures were published. After the manual was published, a committee under the leadership of Peter Doudoroff was established by the Committee on Research of the Federation of Sewage and Industrial Wastes Association—now the Water Pollution Control Federation (Doudoroff 1986). This committee gathered materials from many people conducting toxicity studies with fish and aquatic invertebrates (Doudoroff et al. 1951). The report laid the foundation for the acceptance and later publication of standardized test procedures for the first time in the 11th edition of *Standard Methods for the Examination of Water and Wastewater* (American Public Health Association et al. 1960).

By the late 1950's, the number of agencies conducting aquatic toxicological research had increased substantially.

Fig. 2. The U.S. Fish and Wildlife Service Fish Control Laboratory at the junction of the Black, LaCrosse, and Mississippi rivers was operated in 1959–78, primarily to develop methods for the control of rough fish.

For example, two Fish and Wildlife Service laboratories were established in 1959: the Fish Control Laboratory at LaCrosse, Wisconsin (Fig. 2), under the direction of Robert E. Lennon, and the Fish-Pesticide Laboratory at Denver, Colorado, under the direction of Oliver B. Cope. In 1966, the Fish-Pesticide Laboratory was transferred to Columbia, Missouri. Simultaneously, the capacity of the Biology Section of the U.S. Public Health Service laboratory in Cincinnati to conduct toxicity tests was greatly expanded at the Newtown (Ohio) State Fish Hatchery.

Greatly expanded research facilities and progress in analytical chemistry markedly increased scientists' abilities to detect and measure pollutants and test their toxicity to fish. The development of improved and sophisticated spectrophotometric methods of analysis, including atomic absorption spectroscopy and gas–liquid partition chromatography by A. T. James and A. J. P. Martin (1952, as cited by Ettre 1975), enabled investigators to measure test concentrations of chemicals. Although actual concentrations of many chemicals could be measured, numerous studies continued to be conducted in which only calculated (nominal) concentrations were used (Mayer and Ellersieck 1986).

The Recent Years, 1961–1987

Standardization of test procedures in aquatic toxicity testing has been a primary goal of toxicologists in recent years. The Committee on Methods for Toxicity Tests with Aquatic Organisms (1975), represented by the Environmental Protection Agency, U.S. Fish and Wildlife Service, and private industry, first recognized the importance of common methods among all researchers involved in aquatic toxicity tests. This committee recommended common species, test temperatures, test waters, and procedures for toxicity tests. Reconstituted test water formulations and pH buffer schedules developed at the Fish

Control Laboratory, LaCrosse, Wisconsin (Marking 1969), were adopted by the committee. The standardization work of this committee was further promoted by the American Society for Testing and Materials (ASTM). The work of the 1975 committee was adopted with only format changes by ASTM and published as the standard practice for conducting acute toxicity tests with fishes in 1980. Then, in the 15th (1981) edition of *Standard Methods for Examination of Water and Wastewater*, the American Public Health Association, American Water Works Association, and the Water Pollution Control Federation adopted many of the standards promoted by the 1975 committee, including the use of reconstituted water formulations for standardized tests. Thus, the late 1950's dream of a standardized methodology for acute toxicity tests was fulfilled.

Throughout this period, attempts have been made to better define the biotic and abiotic factors that affect the outcome of toxicity tests. Biotic characteristics include such variables as test species, life stage, nutrition, health of test organisms, and acclimation; abiotic factors include temperature, hardness, alkalinity, and pH (Sprague 1985). Mayer and Ellersieck (1986), in their analysis of more than 4,000 toxicity tests with 410 chemicals (mostly organic pesticides) and 66 species of aquatic animals, found an increase or decrease in LC50 (the concentration estimated to be lethal to 50% of the test organisms after 96 h of exposure) per 10° C change in temperature—similar to that predicted by the Q_{10} concept. However, hardness had little effect on the toxicity of organic chemicals that could not be explained by changes due to pH, even though hardness ions—especially calcium—sometimes greatly influence the toxicity of metals to fish (Hunn 1985; Mance 1987), as first documented by Jones (1938). Although pH affected toxicity in only 20% of the chemicals examined, it caused the greatest average change in toxicity (Mayer and Ellersieck 1986).

Hydrogen ion concentration (pH) influences chemicals in at least three ways. (1) It can change the physical state of the molecule (ionization), which in turn changes its lipid-solubility and thus its bioavailability (Hunn and Allen 1974; Marking 1975). Although changes in lipid-solubility can explain much of the change in toxicity of some chemicals such as 3-trifluoromethyl-4-nitrophenol (Marking and Olson 1975), the uptake and toxicity of ionized molecules have not been fully determined and the mechanism of uptake remains speculative (Allen and Hunn 1986). (2) The pH can influence the rate of degradation (hydrolysis) of the chemical (Mayer and Ellersieck 1986). (3) The pH also affects metal speciation as well as other salts in solution that may determine bioavailability of the metal. Statistical models such as REDEQL-EPAK can be used to estimate the amounts of various species of metal in a particular test water, thus improving the interpretation of toxicity data (Palawski et al. 1985).

Acute toxicity tests can be used for a variety of purposes, one of which is the estimation of the half-life of biological activity of a toxicant (Marking 1972; Marking and Dawson 1972). Two approaches were outlined, one calling for the use of only one species of fish and aged solutions and the other involving aged solutions and several species of fish, each with different sensitivities to the toxic chemical (Marking 1972). The resulting LC50 data can be used to estimate the half-life of biological activity or to calculate a deactivation index. A deactivation index greater than 1.0 indicates loss of biological activity, and an index of less than 1.0 indicates an increase in toxicity of the aged solution over time.

Another application of acute toxicity tests is in on-site toxicity assessment. For example, on-site tests have been used in sea lamprey control in the Great Lakes basin (Howell and Marquette 1962) to establish concentrations of fish toxicants for use in fish control (Burress 1975), to measure the toxicity of effluents (Norberg and Mount 1985), and to monitor water quality (Hall et al. 1985; Susan E. Finger, personal communication). Most tests involve static or static-renewal exposure systems but a few use a flow-through arrangement (Fig. 3).

The acute toxicity of sediments can be assessed by using a liquid phase elutriate test or solid phase tests in which fish are exposed directly to the sediment and water or to water flowing over the sediment, in a modified static test system based on the technique of Fremling (1975). The elutriate or liquid phase testing has shown little promise in defining the toxicity of sediments (Dillon and Gibson 1986). Marking et al. (1984) tested suspended sediments against fish to define the potential toxicity of sediments suspended by commercial boat traffic on the Upper Mississippi River system. Prater and Anderson (1977) used the modified Fremling (1975) assay system to assess the 96-h toxicity of harbor sediments. A further modification of this system was discussed by LeBlanc and Surprenant (1985) in an assessment of toxicity of contaminated freshwater sediments. Sediment toxicity testing remains a subject of active research on the development of methods and the standardization of test protocols.

In the last 15 years, interest has grown in assessing the toxicity of mixtures of chemicals to fish. Marking and Dawson (1975) published a method of assessing the toxicity of mixtures of chemicals by using an additivity index in which additive values were represented by zero,

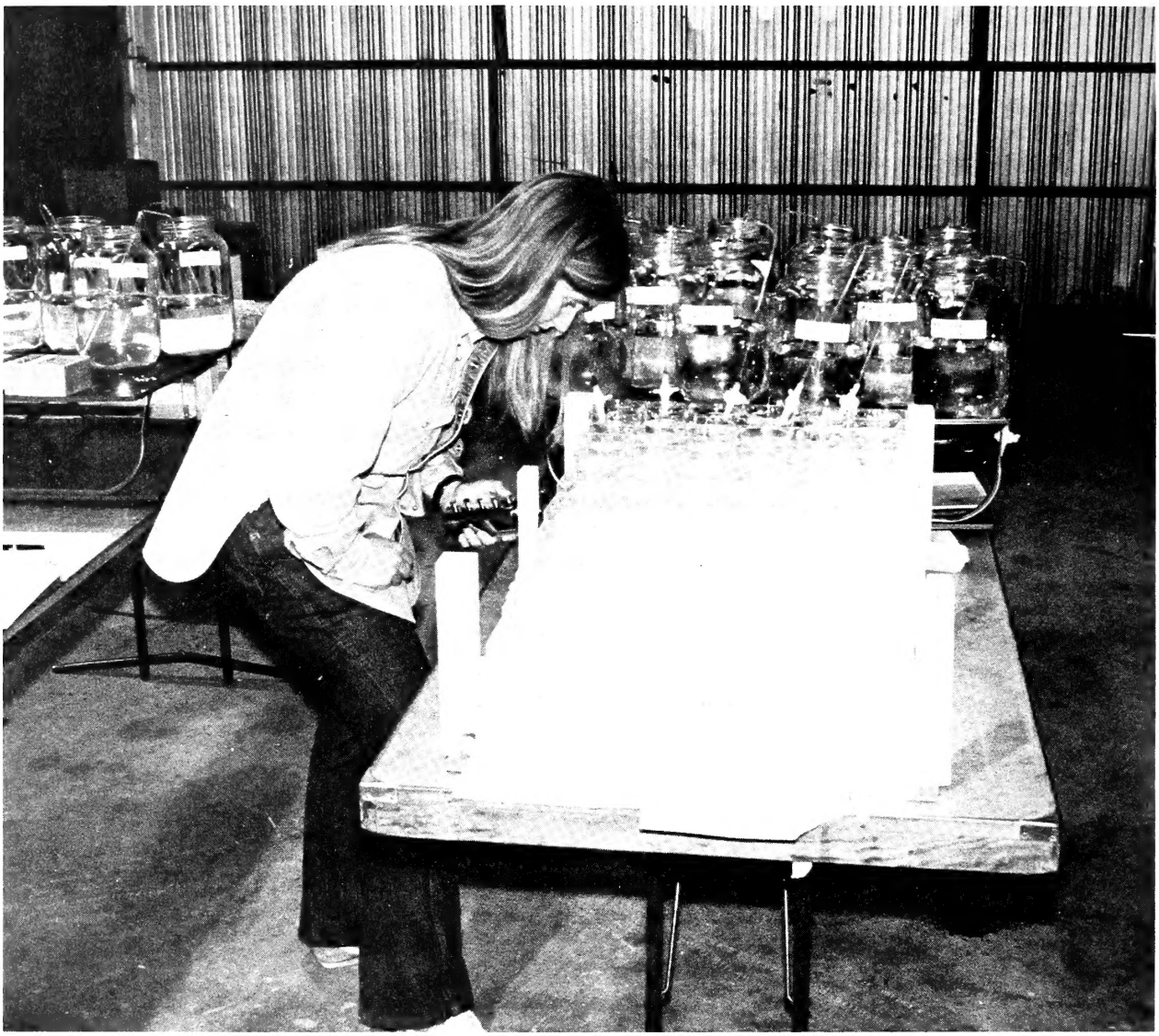

Fig. 3. Chemist evaluating the toxicity of river water to larval striped bass using an on-site flow-through toxicity test system. *Photo by P. Winger.*

greater than additive values by positive numbers, and less than additive values by negative numbers. The method for assessing additive toxicity of chemical mixtures was further defined by Marking (1977, 1985). Könemann (1981) proposed the use of a mixture toxicity index. This scale for measuring the toxicity of mixtures fulfills two requisites he outlined: (1) the scale should give, independently from the number of compounds in the mixture and the ratio between the concentrations, constant values for two reference points—no addition (zero), and concentration addition (1.0); and (2) it should have a logarithmic form because of the log-normal distribution of LC50's. Könemann (1981) proposed the use of the mixture toxicity index for mixtures of two or more chemicals. Broderius and Kahl (1985) demonstrated concentration additive, acute joint toxicity for 27 organic chemicals from 7 different classes, using mixtures of as many as 21 chemicals. Their results are consistent with those of other investigators and suggest that numerous industrial chemicals are likely to kill fish through a similar narcosis type of toxic action.

Also during the last 15 years, data generated from acute toxicity tests have been increasingly used to develop quantitative structure–activity relations (QSAR's) in an effort

to predict the toxicity of pollutants (chemicals) from their physiochemical properties (Lipnick et al. 1985; Hermens 1986; Kaiser 1987). Hermens (1986) suggested that QSAR's will eventually replace range-finding tests and will be used for several other purposes: to set priorities for testing existing compounds, to predict the aquatic toxicity of pesticide products during their development, to classify large numbers of compounds into a limited number of groups, and to estimate the joint toxicity of mixtures.

Data from acute tests are also used to register the uses of various chemicals for fisheries work by the Food and Drug Administration and the Environmental Protection Agency (Meyer and Schnick 1980). The conduct of some acute aquatic toxicity tests now falls under the Good Laboratory Practice Standards (GLPS; Federal Register 1987). The GLPS also apply to studies conducted for the Federal Insecticide, Fungicide and Rodenticide Act and Toxic Substances Control Act. In addition, the use of acute toxicity data plays an integral role in ecological risk assessment procedures (Urban and Cook 1986). Static acute toxicity tests will thus continue to play an essential but limited role in aquatic toxicology in the future.

Table. *Fish toxicity testing (1863–1987): a brief review (year, investigators, significant event, and reference).*

1863	C. Penny and C. Adams	First recorded toxicity tests with fish. More than 400 tests with 71 chemicals in river water and test solutions. (Jones 1964)
1917	E. B. Powers	Recommended the goldfish as a test animal and introduced the concept of measuring survival time versus concentration. (Powers 1917)
1919–30	K. E. Carpenter	Used caged fishes to document toxicity of runoff from lead and zinc mine tailings. In studies with 13 species of freshwater fishes, showed that exposure of fish to lead salts in distilled water resulted in similar symptoms of intoxication. (Carpenter 1930)
1927	D. L. Belding	First published critical analyses of conditions necessary for successful testing of polluting substances against fish. (Belding 1927)
1928	P. Steinmann	First recordings of respiration and heart rate of trout exposed to toxic concentrations of chemicals. (Steinmann 1928)
1937	M. M. Ellis	Classic paper on "Detection and measurement of stream pollution" brought together most of knowledge on mode of action of toxicants. Also includes extensive laboratory toxicity tests with goldfish and *Daphnia magna*. (Ellis 1937)
1938	J. R. E. Jones	Documented the negative effect of calcium on the toxicity of lead and zinc to the stickleback (*Gasterosteus aculeatus*) in soft water. (Jones 1938)
1942		Introduction of DDT as an "ideal" insecticide. (Dunlap 1981)
1948	C. M. Tarzwell	U.S. Public Health Service established Biology Section in Cincinnati, Ohio, laboratory to study water pollution problems. (C. M. Tarzwell, personal communication, 1985)
1950	P. Doudoroff and M. Katz	Critical review of literature on the toxicity of industrial wastes and their components to fish. Parts I and II (Doudoroff and Katz 1950, 1953)

Table. *Continued.*

1951	P. Doudoroff and committee members: Development of standardized criteria for acute toxicity tests began in earnest and culminated in the 1970's with the acceptance of standardized testing criteria. (Doudoroff et al. 1951; P. Doudoroff, personal communication, 1986)
1952–54	E. M. Wood, E. Hollis, and R. E. Lennon: Publication of the largest data set on the toxicity of chemicals to freshwater fish to that time. More than 4,000 chemicals were screened against six species of fish. (Wood 1953; Hollis and Lennon 1954)
1952	A. T. James and A. J. P. Martin: Established gas–liquid partition chromatography as an analytical technique. (Ettre 1975)
1953	V. C. Applegate and coworkers: The screening of chemicals for use in the control of the sea lamprey in the Great Lakes drainage began in 1953 at the Hammond Bay Biological Station. More than 4,000 chemicals were tested before the discovery of the selective lamprey larvicide, 3-trifluoromethyl-4-nitrophenol. (Applegate et al. 1957; Howell et al. 1980)
1959	R. E. Lennon: Opening of the Fish Control Laboratory in LaCrosse, Wisconsin, to investigate physical, chemical and biological methods for the control of nuisance fishes. (R. E. Lennon, personal communication, 1985)
1959	O. B. Cope: Establishment of the Fish-Pesticide Research Laboratory at Denver, Colorado. (O. B. Cope, personal communication, 1985)
1960	C. M. Tarzwell and coworkers: The capacity of the Biology Section of the U.S. Public Health Service Laboratory to do toxicity testing with fish greatly expanded at the Newtown (Ohio) State Fish Hatchery. (Tarzwell 1978)
1960	Standard Bioassay Methods Committee: Publication of "Bioassay methods for the evaluation of acute toxicity of industrial wastes and other substances to fish" in the 11th edition of *Standard Methods*. (American Public Health Association et al. 1960)
1975	Eleven-member committee: Publication of *Methods for Acute Toxicity Tests with Fish, Macroinvertebrates, and Amphibians*. (U.S. Environmental Protection Agency 1975)
1980	Subcommittee E 35.23 on Safety of Aquatic Organisms: Approval and publication of *Standard Practice for Conducting Acute Toxicity Tests with Fishes, Macroinvertebrates, and Amphibians*. (American Society for Testing and Materials 1980)

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